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## INVESTIGATIONS OF THE ETIOLOGY OF INFECTIOUS ABORTION OF MARES AND JENNETS IN KENTUCKY.\*

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Probably no section of the United States has as high-priced horses as the Blue Grass region of Kentucky, the home of the thoroughbred, the standardbred and the saddle horse. It is no uncommon thing for the breeders of this section to pay thousands of dollars for individuals—even the hundred-thousand-dollar mark has been reached. Occasional outbreaks of infectious abortion among the mares are a great source of financial loss to the breeders of these horses. It has been estimated that Kentucky alone has sustained a loss running into the millions of dollars from the results of this disease. There have been seasons when as many as 70 per cent of the mares in some studs aborted, and occasionally a stud is reported where all the mares have slipped. This disease is not confined to Kentucky, for outbreaks have been noted in other states, as well as in Canada. According to Williams,<sup>1</sup> the disease first made its appearance in the United States in 1886, when it appeared in several states of the Mississippi Valley, in which region the breeding of horses had become a very important industry. He states that at that time the malady acquired a very extensive distribution and high degree of virulence.

In this part of the country, mares are bred soon after foaling. If the disease of infectious abortion is present in a stud of mares, most of the slips occur between November 1 and March 1. Close observers state that more abortions occur during the eighth month of pregnancy than at any other period. Mares carrying their foals ten and one-half months usually deliver them alive. We have examined some cases where the abortions occurred in the early stages of gestation. Usually a mare will abort a fetus well along

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<sup>1</sup> *Veterinary Obstetrics*, p. 478.

in gestation with little effort, and generally with no injurious sequelae. Occasionally an individual succumbs from metritis, after aborting.

The discharge from a mare after aborting is of a chocolate color. The veterinarians in this section of the country note a peculiar odor associated with the fetus and fetal membranes of a case of typical abortion. We have found this odor to be more pronounced in some cases than in others, and not only detected on the exterior of the fetus and in the internal organs, but sometimes on the after-birth also. The laymen usually speak of it as a "sour odor." Chemists at this station say it resembles an etheric odor.

The contents of the stomach of the fetus are acid to phenolphthalein at first, but upon standing soon turn to an alkaline reaction. The lungs show areas of hepatization. The spleen usually appears enlarged and covered with petechiae. The mesenteric lymphatic glands are often congested and the liver may show hemorrhagic areas on its surface.

Colts that come alive from a stud of mares affected with abortion are often rheumatic and have swollen joints. This malady is known among breeders of horses as "rheumatism" or "joint ill." Colts affected with "joint ill" appear very lame and walk with difficulty, while in some cases abscesses form at the joints. The hock, knee, ankle, or any joint may be affected, but the articulation at the hock is the usual seat of this trouble. The eyes may be involved, the sight becoming affected, and total blindness may result. In some cases, in a stud afflicted with contagious abortion, colts delivered at the full term of gestation, although they appear strong and healthy when foaled, suddenly sicken and die with a form of pneumonia, while others may become affected with white diarrhea, which usually terminates fatally. A large number of mares in a stud may abort during one year and nearly all deliver live healthy colts the next year.

Ostertag, of Germany, who has investigated the etiology of this disease among mares, found in the heart blood of the fetus, in the thoracic cavity, and in the intestinal tract a streptococcus which usually grew in pairs. He also secured this germ from the fetal membranes. This organism was gram-negative.

In 1890, Kilborne<sup>1</sup> found a short bacillus possessing pathogenic properties in the vagina of a mare which had recently aborted. This mare was one of a number that aborted in a large stud in Pennsylvania. Theobald Smith<sup>2</sup> made a study of this bacillus and



FIG. 1.—Mare and aborted fetus. Abortion caused experimentally by an intravenous injection of 2 c.c. of culture suspension of *B. abortus equinus* in physiological solution. Incubation period 10 days.

decided that morphologically and physiologically it resembled *B. cholera suis*. The characteristics of this bacillus, as it appeared on an agar slant, were a tendency to form wrinkles near the drop of

<sup>1</sup> Bull. No. 3, Bureau of Animal Industry, Washington, 1893, p. 49.

<sup>2</sup> Ibid., 1893, p. 53.

condensation, and the membranous nature of its growth. A pure culture of this bacillus injected intravaginally into both a pregnant mare and cow produced a catarrhal discharge from the vagina for some time, but the mare delivered her foal and the cow her calf, alive.

Much speculation over the cause of the disease has been made by the breeders. The trouble is caused, according to some theories,



FIG. 2.—*Bacillus abortivus equinus*. Plain agar streaked with heart blood of an aborted fetus of a mare. Culture 24 hours old. Actual size.

by the mare eating frost-bitten grass late in the fall, or early in the spring; by the flushing of the system due to eating too much succulent feed; by the presence of malodors, i.e., if a mare aborts in a field and the fetus and afterbirth are not removed at once and the place on the ground from which they were taken covered with dirt, the odor will of itself cause other mares to abort; by the running of mares on the same pasture year after year, or the feeding

of too much salt. Some breeders are of the opinion, however, that the trouble is due to a microbe.

No conclusive results having been obtained on the etiology of this disease, the Animal Husbandry Division of the Kentucky Agricultural Experiment Station feeling that this was a subject vital to the special breeding interests of Kentucky, equipped a laboratory for investigation along this line.

In April, 1912, a preliminary report of these investigations was made,<sup>1</sup> in which it was stated that a bacillus belonging to Subgroup II, of the Colon typhoid group, to which group *B. enteritidis* belongs, had been isolated from the afterbirth, the uterine exudate and internal organs of fetuses of mares and jennets infected with contagious abortion. This organism in most instances was found in abundance. Since that time, these investigations have been continued to determine whether or not this bacillus could be found in an outbreak of the disease in a subsequent year, and if the disease could be produced experimentally with this germ.

#### HISTORY OF THE STUDS.

*Stud No. 1.*—The disease existed in this stud in April, 1911. All five jennets in this stud aborted in quick succession.

*Stud No. 2.*—The disease existed among the mares in this stud in the fall and early winter of 1911; 10 out of 19 mares in one field aborted in a comparatively short time.

*Stud No. 3.*—History incomplete. This stud consisted of a very few mares. One abortion reported.

*Stud No. 4.*—Sixteen out of 30 mares of this stud, in one field, aborted during the late fall of 1912.

*Stud No. 5.*—Twenty-eight mares aborted in different fields on this farm during the late fall and winter of 1912 and the early winter of 1913.

*Stud No. 6.*—History incomplete. A number of mares aborted on this farm during the late fall and winter of 1912.

*Stud No. 1, Case No. 1.*—On June 24, 1911, a fetus and afterbirth were obtained from the fifth and last jennet to abort in this stud. The fetus arrived at the laboratory within half an hour after it was dropped. The fetus was in the eleventh month of gestation. Streaks of blood and contents of the following organs were made on numerous plates of plain agar, standardized neutral to phenolphthalein: heart, liver, spleen, kidneys, stomach, lymphatics, large intestines, small intestines, uterus, and ovaries. Each organ was seared and afterward opened with a sterile lance and one or two loopfuls of the contents streaked on plain agar, as in making streak dilutions. These plates were then incubated 24 hours at 37° C. and examined, with the result that a

<sup>1</sup> E. S. Good, *Ann. Rept. Ky. Agri. Exper. Sta.*, 1912, p. 225.

pure culture in abundance was found on all the plates. Platings from the afterbirth revealed the same organism in abundance. After making an extended study of this organism it was placed in Subgroup II, or Intermediate group of the Colon typhoid group, to which subgroup belong *B. enteritidis* and *B. cholera suis*.

*Stud No. 2, Case No. 1.*—On November 1, 1911, a fetus covered with the fetal membranes from a mare aborting in the eighth month of gestation was brought to the laboratory. The same organism was isolated from all the organs of this fetus and from the afterbirth, as was obtained from the fetus and afterbirth of the aborting jennet in Stud No. 1.

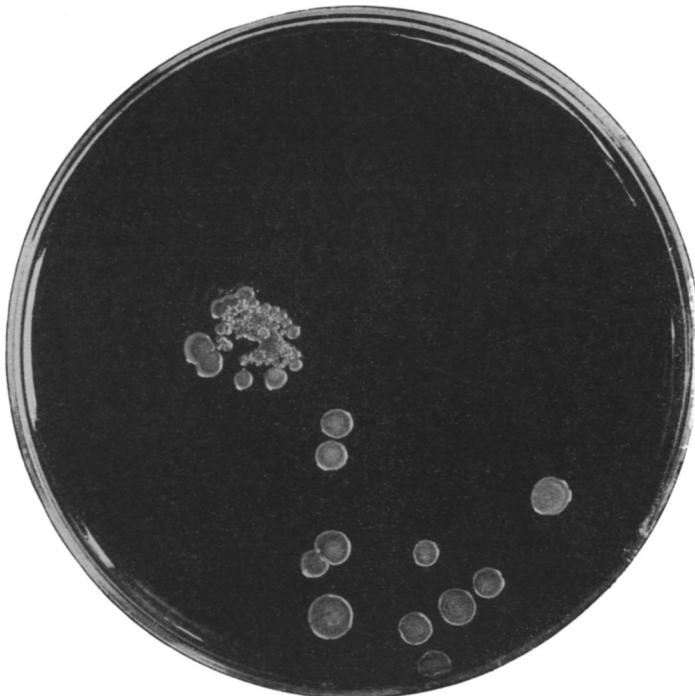


FIG. 3.—Colonies of *Bacillus abortivus equinus* on plain agar. Four days old. Petri dish originally streaked with the uterine exudate of an aborting mare. Actual size.

*Stud No. 2, Case No. 2.*—This mare aborted in the 212th day of gestation. The fetus and afterbirth were destroyed by burning. Material was taken from the uterus the next day and plated, with the result that the same organism was obtained as has been previously mentioned.

*Stud No. 2, Cases No. 3 and 4.*—On November 13, 1911, we learned that two mares in this stud had aborted and the fetuses and afterbirths been burned. On November 16, Dr. J. T. Shannon of Lexington, at our request, visited this place and took some of the contents of the uteruses of these mares under as aseptic conditions

as possible. Plate cultures revealed two organisms, the predominating one being the bacillus obtained before from aborting mares in this stud.

*Stud No. 2, Case No. 5.*—One of the stallions of this stud had a swollen testicle (orchitis) of a chronic type. Pus of this testicle was secured under aseptic conditions and plated, with the result that *B. pseudomonas pyocyaneus* predominated. A few colonies were obtained, however, of the organism associated with infectious abortion in the mares of this stud.

*Stud No. 2, Case No. 6.*—On December 21, 1911, a male fetus, aborted in the eighth month of gestation, was sent to the laboratory. Cultural and microscopical tests showed the internal organs of this fetus to be invaded with the same bacillus before mentioned.

*Stud No. 2, Case No. 7.*—On January 8, 1912, a fetus was taken from a mare about to abort in this stud. The results were negative. The afterbirth of this mare was not secured.

*Stud No. 2, Case No. 8.*—On January 26, 1912, we received a male fetus from a mare aborting in the eighth month of gestation. Streak dilutions, made from the internal organs of this fetus and bits of the afterbirth, gave positive results. A pregnant guinea-pig, inoculated subcutaneously with 4 c.c. of a sterile water suspension of material taken directly from the stomach of the fetus, aborted in 24 hours, and the germ of abortion was isolated from the heart blood and uterus of this pig. The sow had eaten the fetuses, thus giving us no opportunity to determine if the germ could be recovered from them.

*Stud No. 3, Case No. 1.*—On February 9, 1912, we received a fetus of a mare aborting on this farm in the 226th day of gestation. There was dirt and litter on the afterbirth; therefore a portion of it was first washed with tap water, then with 95 per cent alcohol, and then repeatedly with sterile water. Small pieces of it were then cut up finely and streaked over several dishes of agar, with the result that a large number of colonies were obtained of the germ before mentioned as connected with the afterbirth and fetuses of aborting mares. Platings of the internal organs of the fetus were also positive.

*Stud No. 4, Case No. 1.*—On December 20, 1912, we examined the exudate from an aborting mare in this stud, with positive results, both microscopically and culturally.

*Stud No. 5, Case No. 1.*—On December 30, 1912, we examined the exudate from an aborting mare on this farm, with negative results.

*Stud No. 4, Case No. 2.*—The exudate from an aborting mare was examined, January 6, 1913, with positive results.

*Stud No. 5, Case No. 2.*—On February 4, the exudate from an aborting mare was examined with positive results.

*Stud No. 4, Case No. 3.*—The afterbirth and exudate of a mare aborting on February 3, were examined with negative results.

*Stud No. 5, Case No. 3.*—A mare aborted on February 4. On February 5, the afterbirth and exudate were examined with positive results.

*Stud No. 5, Case No. 4.*—On February 10 a mare aborted, and an examination of the uterine exudate gave positive results.

*Stud No. 5, Case No. 5.*—On February 11, the afterbirth of an aborting mare was examined with positive results.

*Stud No. 6, Case No. 1.*—On February 13, we examined the afterbirth and fetus of an aborting mare with positive results.

*Stud No. 5, Case No. 6.*—The afterbirth, uterine exudate and fetus of a mare aborting on February 14, 1913, gave positive results.

*Stud No. 5, Case No. 7.*—The uterine exudate of a mare aborting on February 17 gave positive results.

*Stud No. 4, Case No. 4.*—The exudate from a mare aborting on February 17 gave positive results.

*Stud No. 5, Case No. 8.*—The uterine exudate from a mare aborting February 21 gave positive results.

*Stud No. 5, Case No. 9.*—Uterine exudate of a mare delivering a live foal, but showing a characteristic chocolate colored discharge from the genitals, was examined on March 4 with positive results.

In both seasons, we examined a number of fetuses and afterbirths of mares, supposed to have aborted from accident or multiparous pregnancy, in studs where infectious abortion did not exist. In nearly every instance when examined soon after being aborted, the internal organs of these fetuses were found to be sterile. The bacillus of abortion in mares was not found in any of this material. *B. coli communis* was found associated in large numbers with two small aborted embryos.

In all these investigations, the uterine exudate and material from the fetuses of aborting mares in infected studs have been subjected to the cultural conditions needed for the growth of *B. abortus* Bang, the organism causing the disease in the cow, but in no instance at this laboratory have we found that germ, though we have repeatedly isolated it from the uterine exudates and fetuses of aborting cows.<sup>1</sup> Neither has the streptococcus, mentioned by Ostertag as being associated with infectious abortion in mares, been observed. Many plates streaked with material were incubated under anaerobic conditions, with no further developments.

During the summer of 1912, Dr. Willis Wilson of the state of Washington notified this laboratory that he had found a diplococcus in abundance in the afterbirth of an aborting mare in his section of the country, where the disease of abortion among mares was existing. A culture sent to this laboratory by Dr. Wilson revealed the same organism that had been isolated at this laboratory from aborting mares. It conformed to some strains isolated here, in that the germ had a tendency to stain at the ends, thus making it appear like a diplococcus. This tendency is sometimes noted in

<sup>1</sup> *Bull. No. 165, Ky. Agri. Exper. Sta., 1912, p. 225.*

examining old cultures. The organism corresponded to all the cultural characteristics of the strains isolated at this laboratory. It also behaved identically in agglutination tests.

#### INOCULATION EXPERIMENTS.

On July 23, 1912, an intravenous injection of a suspension of this organism into the ear vein of a rabbit produced death in 1 hour. Subcutaneous inoculations of contents of a fetal stomach produced

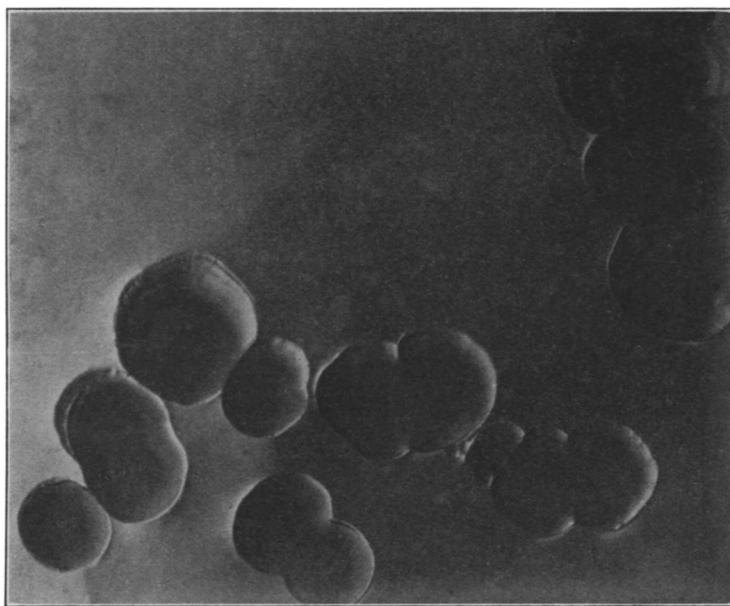


FIG. 4.—*Bacillus abortus equinus*; surface colonies.  $\times 4$ .

abortion in the guinea-pig in about 36 hours. On Feb. 13, 1913, 4 c.c. of a broth suspension of this organism injected intravenously into each of two hogs produced lassitude, loss of appetite, and a decided rise of temperature. The hogs entirely recovered, however, in three or four days' time.

On March 6, 1912, an intravenous injection of a similar amount of the organism in a pregnant ewe produced abortion in 72 hours. The organism was isolated from all the internal organs of the fetus and from the afterbirth of the ewe.

On February 15, 1913, a subcutaneous injection of 4 c.c. of a physiological salt suspension of the organism in a pregnant ewe did not produce abortion.

On May 10, 1912, an intravenous injection of 20 c.c. of a physiological salt suspension of the organism produced abortion in a pregnant 250-pound sow in 41 hours. The organism was recovered from the afterbirth but not from the fetuses.

On February 7, 1912, a supposedly pregnant mare was slowly injected intravenously with 10 c.c. culture suspension and found afterward not to be in foal. This injection nearly proved fatal to the mare, as the following day her temperature rose to 108 degrees. She soon recovered, however.

On February 28, 1913, 10 different strains of the bacillus isolated from aborting mares were washed off agar slants with physiological salt solution and 2 c.c. injected intravenously into a pregnant mare. The preinjection temperature of this mare was 99.8° F. At the end of five hours it registered 103.8° F. At the end of 21 hours after inoculation her temperature was again normal and continued normal until she aborted. On March 10, this mare aborted without any seeming inconvenience. The afterbirth and internal organs of the fetus were typical of cases where natural infection existed. The organism was found in pure culture and in abundance in the heart, liver, lungs, spleen, kidneys, stomach, lymphatics, intestines, and testicles of this fetus. The germ was also isolated in abundance from the afterbirth and from the contents of the uterus. The history of this mare was known. She was eight years of age, had delivered four normal foals, and had never aborted. One of the writers took care of the mare after treatment, and at no time after the second day of the inoculation did she show any abnormal symptoms or temperature. She gave no indication at any time that she would abort. Soon after the mare aborted the genital tract was flushed with a one per cent solution of creolin, and on three subsequent days with a saturated solution of boric acid. The mare was somewhat depressed for two days after aborting. Her appetite, however, was nearly normal. After the third day, she appeared normal in every way and took the stallion the eighth day after aborting. There was some chocolate colored discharge

from the vagina for six days after slipping. Cultures made from this discharge, taken from the vagina, revealed the abortion bacillus up to the fifth day after aborting. From that time on, all tests were negative, as far as securing a culture of this organism.

MORPHOLOGICAL AND CULTURAL CHARACTERISTICS OF THE ORGANISM.

*Morphology and Staining.*—In the tissues, the organism resembles a coccus and a short plump bacillus with rounded ends measuring 0.3 to 0.5  $\mu$  in width and 0.5 to 1.0  $\mu$  in length. When

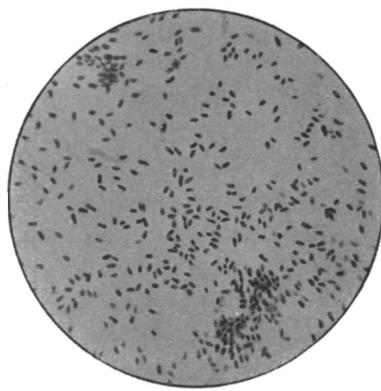


FIG. 5.—*Bacillus abortivus equinus*.  $\times 1000$ .



FIG. 6.—*Bacillus abortivus equinus*.  $\times 1800$ .

cultivated, there is some increase in size. The organism stains somewhat irregularly. Some stained preparations show many bacilli staining at the ends. In routine work at this laboratory, we stain with carbol-fuchsin for from one to two seconds. The organism is gram-negative.

*Sporulation.*—No spores produced.

*Motility.*—It is slightly motile when the germ is examined from the drop of condensation of an 18-hour-old agar slant culture.

*Petri-dish culture.*—Surface colonies 24 hours old are round, measuring from a pin point to one-fourth of one millimetre in diameter. To the naked eye, these colonies are glistening, homogeneous and transparent to direct light, and of a bluish-gray color to reflected light. When incubated for two or three days at 37° C.,

the colonies show a marked contrast to a young culture, as they are dull, granular, and of a grayish color. If the colonies are few in number and well separated, they sometimes attain a size of 2-5 mm. in diameter and may become irregular in outline. The edges of some colonies appear as if they had been pressed flat, other colonies assume crater forms, and still others may be concentrically ringed. Occasionally, a typical rose-shaped colony is noted. Large colonies of some

strains have a decided tendency to wrinkle. In attempting to remove a portion of one of these colonies with a platinum needle, it is found to be tenacious and bits of the same are obtained with some difficulty. A whole colony will at times slide along on the surface of the medium, if pushed with a needle. If the surface colonies are numerous, they increase but little in size, the borders are regular and the contents granular and homogeneous. All the foregoing description pertains to colonies obtained by streak dilutions of material direct from the tissues.

FIG. 7.—*B. abortivus equinus*; broth culture.

*Gelatin*.—Does not liquefy gelatin; stab culture filiform.

*Broth*.—After a few days' time, if incubated at 37° C., a whitish, wrinkled pellicle forms at the surface, which, upon shaking slightly, settles to the bottom of the tube en masse.

*Milk*.—Does not coagulate milk even on being incubated 10 days, and the end of reaction is alkaline to litmus.

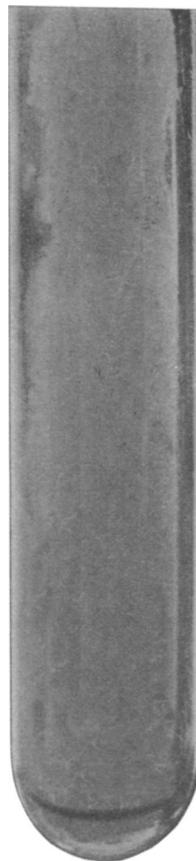
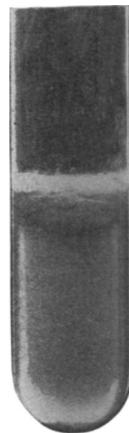


FIG. 8.—*B. abortivus equinus*. Streak on plain agar. Actual size.

*Agar slants.*—Streaks of the bacillus on agar slants are very characteristic, in that after the tubes are incubated for a day or two the growth becomes dull and parchment like, and usually develops thin wrinkles near the drop of condensation. The drop of condensation is covered with a film of growth.

*Physiology.*—The organism is aerobic and facultative anaerobic. Dextrose, sorbit, dulcit, mannite, xylose, arabinose, and raffinose are fermented with the production of both acid and gas. While we have reported that this organism does not ferment lactose and saccharose, we have since determined that most of our strains now produce a trace of acid and gas in broth containing one per cent of these sugars. Adonit, sorbose, and rhamnose are not fermented. Our investigations, so far, tend to show that while sorbose yields no gas, a slight amount of acid is produced. All broth to which sugars had been added was made from an infusion of beef. The beef infusion was rendered free from muscle sugar according to the usual technic, by inoculation with *B. coli communis*. The germ produces six per cent of gas in Jackson's bile tube in 48 hours.

Indol was not produced.

Although these fermentation tests were somewhat extensive, we wish to state that we do not deem the results tabulated as exactly typical of all strains of this organism. More work is now being done along this line.

#### AGGLUTINATION AND COMPLEMENT FIXATION TESTS.<sup>1</sup>

Tables 1 and 2 give the results of agglutination and complement fixation tests of blood from mares which had aborted, as well as blood from non-aborting mares in non-infected studs.

On March 10, 1913, blood was drawn from 13 of 28 aborting mares (Nos. 1 to 13 in Stud No. 5, Tables 1 and 2). Darkness prevented blood being taken from the entire 28 at that time. These mares aborted at different intervals between December 1, 1912, and February 25, 1913. Numbers 19 and 19a represent the test of blood of the mare before and after abortion was produced experimentally. The second blood drawing was taken 8 days after aborting.

<sup>1</sup> Dr. F. M. Surface of this station kindly ran the complement fixation test for the writers.

As can be seen from Table 1, the blood serum of aborting mares (Nos. 1 to 13) agglutinated the bacillus in dilutions varying from

TABLE 1.\*  
SHOWING AGGLUTINATION TESTS WITH THE BACILLUS FOUND CONNECTED WITH INFECTIOUS ABORTION  
IN MARES.  
Final Reading After 60 Hours' Incubation.

Source of Blood Serum. Number	I-200	I-300	I-500	I-800	I-1000	I-1500	I-2000	I-3000	Check
1. Aborting Mare.....	+	+	+	-	o	o	o	o	o
2. " "	+	+	+	+	+	+	+	+	o
3. " "	+	+	+	+	+	+	+	+	o
4. " "	+	+	+	+	+	o	o	o	o
5. " "	+	+	+	+	+	+	+	+	o
6. " "	+	+	+	+	+	+	+	+	o
7. " "	+	+	+	+	+	+	+	-	o
8. " "	+	+	+	+	+	o	-	o	o
9. " "	+	+	+	+	+	+	-	-	o
10. " "	+	+	+	+	+	+	-	-	o
11. " "	+	+	+	+	+	+	+	+	o
12. " "	+	+	+	+	+	+	o	o	o
13. " "	+	+	+	+	+	+	o	o	o
14. Normal.....	+	-	o	o	o	o	o	o	o
15. " "	+	-	o	o	o	o	o	o	o
16. " "	+	o	o	o	o	o	o	o	o
17. " "	+	o	o	o	o	o	o	o	o
18. " "	-	o	o	o	o	o	o	o	o
19. Before aborting.....	+	o	o	o	o	o	o	o	o
19a. After aborting.....	Gave plus agglutination		I:5000, minus at I:8000						

\* In the table the sign (+) indicates a complete agglutination; the sign (-) indicates that the reaction has progressed considerably, but not a clearing up of the turbidity of the antigen; and the sign (o) indicates no change.

TABLE 2.\*  
COMPLEMENT FIXATION TESTS.

NUMBER OF HORSE	AMOUNT OF INACTIVATED HORSE SERUM				
	0.2 C.C. Control without Antigen	0.1 C.C.	0.05 C.C.	0.03 C.C.	0.01 C.C.
1.....	++++	o	++	++++	++++
2.....	++++	o	o	o	o
3.....	++++	o	o	o	o
4.....	++++	o	o	o	+
5.....	++++	o	o	o	o
6.....	++++	o	o	o	o
7.....	++++	o	o	o	o
8.....	++++	o	o	o	o
9.....	++++	o	o	o	o
10.....	++++	o	o	o	o
11.....	++++	+++	+	+	+
12.....	++++	o	o	o	o
13.....	++++	o	o	o	o
14.....	++++	++++	++++	+++	+++
15.....	++++	++++	++++	++	++
16.....	++++	++++	++++	++++	++++
17.....	++++	++++	++++	++++	++++
18.....	++++	++++	++++	++++	++++
19.....	++++	++++	++++	++	++
19a.....	++++	++	o	o	o

\* In this table, ++++ means complete hemolysis; ++ nearly complete solution; + partial hemolysis; + a trace of hemolysis; while o indicates complete fixation of the complement.

1:500 in No. 1, to 1:3,000 in Nos. 2, 3, and 11. No. 19a, in which abortion was produced experimentally, gave complete agglutination 1:5,000 and considerable reaction in a dilution of 1:8,000. The blood from non-infected horses (Nos. 14 to 18, inclusive, Tables 1 and 2) agglutinates 1:200 with considerable reaction, in two instances at 1:300.

Dr. Surface comments on Table 2 as follows:

"1. Each of the first 13 mares shows some fixation of the complement. Mares No. 1 and 11 do not show as marked fixation as the others.

"2. Mares No. 14, 15, and 16 came from a farm where there had been no abortion. Nos. 14 and 15 show some tendency to inhibit the hemolysis, but these could not be regarded as definite fixations. Likewise, Nos. 17, 18, and 19, horses which had never been exposed to the disease, show no reaction.

"3. Thus, all the 13 mares which aborted respond positively to the fixation test, while the five mares from non-infected farms react negatively.

"4. Mare No. 19, when tested before inoculation, showed no definite fixation, although small amounts of serum appeared to inhibit the hemolysis to a slight extent. Seventeen days after inoculation (No. 19a), she showed marked fixation of the complement."

As this paper was being written, Dr. Surface called the attention of the writers to an article<sup>1</sup> by Professor Dr. D. A. de Jong of Leiden, Holland. In October, 1911, his attention was called to an outbreak of infectious abortion in one of the provinces of his country. He stated that the abortions came without alarming previous symptoms and with no injurious sequelae, the mares being able to work soon after aborting. Dr. de Jong secured a culture of a short, oval bacillus in abundance from the internal organs of two aborted fetuses, as well as from the fetal membranes. He found the bacillus in pure culture.

Dr. de Jong placed the bacillus in the Paratyphus *B. enteritidis* group, from the following characteristics: (1) bacillus motile; (2) gelatin not liquefied; (3) milk not coagulated; (4) grape sugar fermented; (5) saccharose fermented; (6) milk sugar not fermented; (7) litmus milk turned first red, then blue; (8) decolorized neutral red with florescence; (9) no indol formed. He called attention to the tendency of a streak on agar to wrinkle near the drop of condensation.

Dr. de Jong was also able to produce abortion experimentally in mares, by intravenous injection of a culture suspension, the incubation period being 11 days in one instance, and 30 days in another instance. The organism was recovered from the fetus and afterbirth in each instance. He was able, also, to produce the disease by feeding, the incubation period being 13 days, and the organism was recovered from the fetus and afterbirth.

#### SUMMARY.

1. Infectious epizootic abortion is noted from time to time among the mares of this region as well as in other parts of the United States.

<sup>1</sup> *Centralbl. f. Bakteriol.*, 1912, I Orig., 67, p. 148.

2. The etiology of the disease of infectious abortion in the mare, until recently, has been indefinite.

3. From the afterbirths, fetuses and uterine exudates in five studs of aborting mares and one stud of aborting jennets, this laboratory has isolated a germ belonging to Subgroup II of the Colon typhoid group, to which subgroup belong *B. cholera suis* and *B. enteritidis*.

4. In our investigations, *B. abortus* Bang has not been found associated with the disease of infectious abortion in the mare, although we have often isolated the same from aborting cows. Neither has the streptococcus, observed by Ostertag, been found.

5. While we place the bacillus isolated at this laboratory in the same group as *B. enteritidis* and *B. cholera suis*, it differs somewhat in cultural characteristics, and serum immune to the bacillus obtained from the mare does not agglutinate either *B. enteritidis* or *B. cholera suis*.

6. As it is evident that the etiological factors of the disease of infectious abortion of the cow and of the mare are different, we would suggest that the bacillus causing abortion in the mare be differentiated from *B. abortus* Bang by naming it *B. abortivus equinus*.

7. From our investigations, normal horse serum agglutinates *B. abortivus equinus* in a dilution of 1:200, occasionally 1:300, while the serum of infected animals agglutinates from 1:500 to 1:5,000.

8. The results of the work of Dr. F. M. Surface show that the serum of infected animals fixes the complement.

9. A ewe and a sow, injected intravenously, and guinea-pigs, subcutaneously, with pure cultures of this germ, aborted and the organism was recovered from the uterine exudates.

10. An injection of two cubic centimeters of physiological salt suspension of *B. abortivus equinus* in a pregnant mare caused abortion in 10 days, with typical symptoms of natural infection. The organism was recovered in abundance from all the internal organs of the fetus, as well as the fetal membranes and uterus of the mare.